

## AMENDMENTS TO THE SPECIFICATION:

Please amend the paragraph on page 2, lines 22-28 as follows:

Accordingly, the invention provides a polypeptide which:

- a) has maltogenic amylase activity;
- b) has at least 70 % identity to [SEQ ID NO: 1] SEQ ID NO: 2;
- c) has optimum maltogenic amylase activity in the range pH 3.5-7.0 (preferably 4-5.5), and
- d) shows a residual maltogenic amylase activity of at least 25 % after incubation with 1 mM  $\text{Ca}^{++}$  at pH 4.3, 80°C for 15 minutes.

Please amend the paragraph on page 4, lines 1-6 as follows:

A particularly preferred maltogenic alpha-amylase is the amylase cloned from *Bacillus* as described in EP 120 693 (hereinafter referred to as Novamyl). Novamyl has the amino acid sequence set forth in amino acids 1-686 of [SEQ ID NO: 1] SEQ ID NO: 2. Novamyl is encoded in the gene harbored in the *Bacillus* strain NCIB 11837 which has the nucleic acid sequence set forth in SEQ ID NO:1. The three-dimensional structure of Novamyl is described below.

Please amend the paragraph on page 4, lines 10-11 as follows:

- ii) an amino acid sequence having at least 70 % identity to [SEQ ID NO: 1] SEQ ID NO: 2, preferably at least 80 % or 90 %, e.g. 95 % or 98 %,

Please amend the paragraph on page 4, lines 20-22 as follows:

- v) a sequence of five amino acids corresponding to Pro-Ala-Gly-Phe-Ser in a position equivalent to residues 191-195 in the amino acid sequence shown in [SEQ ID NO: 1] SEQ ID NO: 2; and

Please amend the paragraph on page 5, lines 14-19 as follows:

The structure of said maltogenic alpha-amylase is made up of five globular domains, ordered A, B, C, D and E. The domains can be defined as being residues 1-132 and 204-403 for Domain A, residues 133-203 for Domain B, residues 404-496 for Domain C, residues 497-579 for Domain D, and residues 580-686 for Domain E, wherein the numbering refers to the amino acid sequence in [SEQ ID NO: 1] SEQ ID NO: 2. Features of Domains A, B, and C of particular interest are described below.

Please amend the paragraph on page 6, lines 7-13 as follows:

Domain B, also referred to as loop 3 of the (beta/alpha) 8 barrel, in comprises amino acid residues 133-203 of the amino acid sequence shown in [SEQ ID NO:1] SEQ ID NO: 2. The structure is partially homologous to the structure of Domain B in CGTases, the most striking difference being the presence of a five amino acid insert corresponding to positions 191-195 in the amino acid sequence shown in [SEQ ID NO: 1] SEQ ID NO: 2 which is not found in the CGTases. This insert is spatially positioned close to the active site residues and in close contact to the substrate.

Please amend the paragraph on page 6, lines 15-19 as follows:

Domain C in Novamyl comprises amino acid residues 404-496 of the amino acid sequence shown in [SEQ ID NO: 1] SEQ ID NO: 2. Domain C is composed entirely of  $\beta$ -strands which form a single 8-stranded sheet structure that folds back on itself, and thus may be described as a  $\beta$ -sandwich structure. One part of the  $\beta$ -sheet forms the interface to Domain A.

Please amend the paragraph on page 7, lines 4-9 as follows:

Parts of the loop discussed above in the context of domains A and B are of particular interest for substrate interaction and active site reactivity. In particular, in domain A, residues 37-45 in loop 1, residues 261-266 in loop 5, residues 327-330 in loop 7 and residues 370-376 in loop 8; in domain B, residues 135-145 in loop 3, residues 173-180 and 188-196 in loop 3, wherein residue positions correspond to the amino acids in the amino acid sequence in [SEQ ID NO: 1] SEQ ID NO: 2.

Please amend the paragraph on page 7, lines 10-15 as follows:

Without being limited to any theory, it is presently believed that binding between a substrate and an enzyme is supported by favorable interactions found within a sphere of 4 to 6 Å between the substrate molecule and the enzyme, such as hydrogen bonds and/or strong electrostatic interaction. The following residues of Novamyl [(SEQ ID NO:1)] (SEQ ID NO: 2), are within a distance of 6 Å of the substrate HEX and thus believed to be involved in interactions with said substrate:

Please amend the paragraph on page 9, lines 10-13 as follows:

The variants of the invention may comprise additional modifications in addition to the modifications described herein. The variants preferably have an amino acid having more than 70 % identity with [SEQ ID NO: 1] SEQ ID NO: 2, preferably more than 80 %, particularly more than 90 %, especially more than 95 %, e.g. more than 98 %.

Please amend the paragraph on page 10, lines 3-6 as follows:

In a preferred embodiment, the variant of a maltogenic alpha-amylase having an altered pH dependent activity profile as compared to the parent maltogenic alpha-amylase comprises a modification of an amino acid residue corresponding to one or more of the following residues of the amino acid sequence set forth in [SEQ ID NO: 1] SEQ ID NO: 2:

Please amend the paragraph on page 10, lines 12-14 as follows:

In more preferred embodiment, the variant comprises a modification corresponding to one or more of the following modifications in the amino acid sequence set forth in [SEQ ID NO: 1] SEQ ID NO: 2:

Please amend the paragraph on page 11, lines 6-8 as follows:

The amino acid residues found within a distance of 10 Å from the Ca<sup>2+</sup> binding sites of the maltogenic alpha-amylase with the amino acid sequence set forth in [SEQ ID NO:1] SEQ ID NO: 2 were determined as described in Example 2 and are as follows:

Please amend the paragraph on page 12, lines 19-22 as follows:

In a preferred embodiment, the variant of a maltogenic alpha-amylase having an altered Ca<sup>2+</sup> binding as compared to the parent maltogenic alpha-amylase comprises a substitution of an amino acid residue corresponding to one or more of the following residues of the amino acid sequence set forth in [SEQ ID NO: 1] SEQ ID NO: 2:

Please amend the paragraph on page 12, lines 27-29 as follows:

In more preferred embodiment, the variant of a maltogenic alpha-amylase comprises a substitution corresponding to one or more of the following substitutions in the amino acid sequence set forth in [SEQ ID NO: 1] SEQ ID NO: 2:

Please amend the paragraph on page 13, lines 1-3 as follows:

In another preferred embodiment of the invention with respect to altering the  $\text{Ca}^{2+}$  binding of a maltogenic alpha-amylase the partial sequence N28-P29-A30-K31-S32-Y33-G34 as set forth in [SEQ ID NO: 1] SEQ ID NO: 2 is modified.

Please amend the paragraph on page 13, lines 11-17 as follows:

The maltogenic alpha-amylase having the amino acid sequence shown in [SEQ ID NO: 1] SEQ ID NO: 2 may be stabilized by the introduction of one or more interdomain disulfide bonds. Accordingly, another preferred embodiment of the present invention relates to a variant of a parent maltogenic alpha-amylase which has improved stability and at least one more interdomain disulfide bridge as compared to said parent, wherein said variant comprises a modification in a position corresponding to at least one of the following pairs of positions in [SEQ ID NO: 1] SEQ ID NO: 2:

Please amend the paragraph on page 13, lines 22-25 as follows:

Another preferred embodiment of the invention relates to a variant of a parent maltogenic alpha-amylase which has an improved stability and an altered interdomain interaction as compared to said parent, wherein said variant comprises a substitution in a position corresponding at least one of the following sets of positions in [SEQ ID NO: 1] SEQ ID NO: 2:

Please amend the paragraph on page 14, lines 5-8 as follows:

Another preferred embodiment of the invention relates to a variant of a parent maltogenic alpha-amylase which has an improved stability and one or more salt bridges as compared to said parent, wherein said variant comprises a substitution in a position corresponding at least one of the following sets of positions in [SEQ ID NO: 1] SEQ ID NO: 2:

Please amend the paragraph on page 14, lines 10-12 as follows:

In a more preferred embodiment, the variant of a maltogenic alpha-amylase comprises a substitution corresponding to the following substitutions in the amino acid sequence set forth in [SEQ ID NO: 1] SEQ ID NO: 2:

Please amend the paragraph on page 14, lines 14-17 as follows:

Another embodiment of the invention relates to a variant of a parent maltogenic alpha-amylase which has an improved stability and wherein said variant comprises a

substitution in a position corresponding at least one of the following sets of positions in [SEQ ID NO:1] SEQ ID NO: 2:

Please amend the paragraph on page 14, lines 21-23 as follows:

In a more preferred embodiment, the variant of a maltogenic alpha-amylase comprises a substitution corresponding to one or more of the following substitutions with proline in the amino acid sequence set forth in [SEQ ID NO: 1] SEQ ID NO: 2:

Please amend the paragraph on page 14, lines 28-33 as follows:

Analogously, it may be preferred that one or more histidine residues present in the parent maltogenic alpha-amylase is or are substituted with a non-histidine residues such as Y, V I, L, F, M, E, Q, N, or D. Accordingly, in another preferred embodiment, the variant of a maltogenic alpha-amylase comprises a substitution of an amino acid residue corresponding to one or more of the following residues of the amino acid sequence set forth in [SEQ ID NO: 1] SEQ ID NO: 2:

Please amend the paragraph on page 14, lines 35-37 as follows:

In a more preferred embodiment, the variant of a maltogenic alpha-amylase comprises a substitution corresponding to one or more of the following substitutions in the amino acid sequence set forth in [SEQ ID NO: 1] SEQ ID NO: 2:

Please amend the paragraph on page 15, lines 1-5 as follows:

It may be preferred that one or more asparagine or glutamine residues present in the parent maltogenic alpha-amylase is or are substituted with a residue lacking the amide on the side chain. Accordingly, in another preferred embodiment, the variant of a Novamyl-like comprises a substitution of an amino acid residue corresponding to one or more of the following residues of the amino acid sequence set forth in [SEQ ID NO: 1] SEQ ID NO: 2:

Please amend the paragraph on page 15, lines 10-12 as follows:

In more preferred embodiment, the variant of a maltogenic alpha-amylase comprises a substitution corresponding to one or more of the following substitutions in the amino acid sequence set forth in [SEQ ID NO: 1] SEQ ID NO: 2:

Please amend the paragraph on page 15, lines 23-26 as follows:

Another embodiment of the invention relates to a variant of a parent maltogenic alpha-amylase which has improved stability and improved hydrogen bond contacts as compared to said parent, wherein said variant comprises a modification in a position corresponding to one or more of the following positions in [SEQ ID NO: 1] SEQ ID NO: 2:

Please amend the paragraph on page 17, lines 3-6 as follows:

In a preferred embodiment, the variant of a maltogenic alpha-amylase, in order to fill, either completely or partly, cavities located internally in the structure, comprises a substitution of an amino acid residue corresponding to one or more of the following residues of the amino acid sequence set forth in [SEQ ID NO: 1] SEQ ID NO: 2:

Please amend the paragraph on page 17, lines 15-17 as follows:

In a more preferred embodiment, the variant of a maltogenic alpha-amylase comprises one or more substitutions corresponding to the following substitutions in the amino acid sequence set forth in [SEQ ID NO: 1] SEQ ID NO: 2:

Please amend the paragraph on page 18, lines 24-27 as follows:

Accordingly, another aspect of the invention relates to a variant of a parent maltogenic alpha-amylase which has an altered substrate binding site as compared to said parent, which variant comprises a modification in a position corresponding to one or both of the following positions in [SEQ ID NO: 1] SEQ ID NO: 2:

Please amend the paragraph on pages 19, lines 1-2 as follows:

variants comprise a modification at one or more positions corresponding to the following amino acid residues in [SEQ ID NO:1] SEQ ID NO: 2:

Please amend the paragraph on page 19, lines 5-6 as follows:

In a more preferred embodiment, the variant comprises one or more modifications corresponding to the following in [SEQ ID NO: 1] SEQ ID NO: 2:

Please amend the paragraph on page 20, lines 19-21 as follows:

The variants of the invention have an amino acid identity with amino acids 1-686 of [SEQ ID NO: 1] SEQ ID NO: 2 of at least 70 %, preferably at least 80 %, e.g. at least 90 %, particularly at least 95 % or at least 98 %.

Please amend the paragraph on page 25, lines 10-13 as follows:

For region-specific random mutagenesis with a view to improving the stability of calcium binding of a parent maltogenic alpha-amylase, codon positions corresponding to the following amino acid residues from the amino acid sequence set forth in [SEQ ID NO: 1] SEQ ID NO: 2 may appropriately be targeted:

Please amend the paragraph on page 25, lines 25-31 as follows:

With a view to achieving improved binding of a substrate, i.e., improved binding of a carbohydrate species, such as amylose or amylopectin, by a maltogenic alpha-amylase variant with a modified, e.g. higher, substrate specificity and/or a modified, e.g. higher, specificity with respect to cleavage, i.e. hydrolysis, of the substrate, it appears that the following codon positions in the following regions of the amino acid sequence shown in [SEQ ID NO: 1] SEQ ID NO: 2, may particularly appropriately be targeted for modification by region-specific mutagenesis:

Please amend the paragraph on page 25, lines 33-35 as follows:

For region-specific random mutagenesis with a view to altering the substrate specificity and/or the pH dependent activity profile, the following regions of [SEQ ID NO: 1] SEQ ID NO: 2 may be targeted: 70-97, 174-198.